Melanoma-Associated Antigens in Deparaffinized Tissue Sections

To the Editor:
Melanoma-associated antigens (MAA) are a group of molecules which are expressed by tumor cells in malignant melanoma (MM) [1,2]. Ideally, MAA are expected to be absent in normal melanocytes and in proliferating benign pigment cells. Over the last decade many monoclonal antibodies against MAA have been developed [1,2]. Although the available monoclonal antibodies to MAA are not of absolute specificity, some of them are of potential diagnostic, therapeutic, and investigational use in MM [3,4]. The substrates studied so far include cultured melanoma cells and frozen sections of MM. While frozen sections of MM are often impractical to obtain, formalin-fixed and paraffin-embedded specimens are readily accessible.

To determine whether antigenic sites could be detected in paraffin-embedded sections of MM cells, 2 commercially available monoclonal antibodies, p97a and gp240 (Hybritech Inc., California) were utilized in the present investigation, using the standard avidin-biotin complex (ABC) immunoperoxidase (IP) technique [5]. Paraffin-embedded sections of lentigo maligna, superficial spreading MM, nodular MM, and metastatic nodules of MM as well as those of nevocellular (junctional, intradermal, and compound), halo, blue, irritated, and dysplastic nevi were processed. Duplicate paraffin-embedded sections were predigested with trypsin prior to IP processing. Frozen sections of cutaneous MM and metastatic MM to lymph nodes served as positive controls. Frozen sections of normal human skin and tonsils served as negative controls.

Positive control slides exhibited strong staining for MAA with both p97a and gp240 antibodies. Frozen sections of benign nevocellular nevi exhibited positive staining with p97a in nevus cells located in the periphery of the nests. The staining pattern with gp240 was even less specific and, in addition to the nevus cells, included the entire epidermal basal layer. Formalin-fixed and paraffin-embedded sections, however, did not show any specific or nonspecific binding with p97a and gp240 monoclonal antibodies. Predigestion of the paraffin-embedded section with trypsin, to break the aldehyde bonds [6], failed to unmask the antigenic sites.

These results indicate that the ABC IP technique, even with trypsin predigestion, is not sensitive enough to detect MAA by p97a and gp240 in deparaffinized sections of MM.

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Cell Proliferation in Normal and Psoriatic Epidermis

To the Editor:
We have read with interest the recent papers of Weinstein and his colleagues on cell proliferation in normal and psoriatic epidermis [1,2]. These authors have shown, using a direct method, that the cell cycle time (T_c) in psoriasis is about 36 h and have demonstrated rather convincingly that the growth fraction in the psoriatic lesion approaches unity. Further, their figures for the sizes of the differentiated and proliferative compartments are in agreement with our own data obtained by flow cytometric analysis of keratinocytes stained with appropriate monoclonal antibodies [3,4].

However, we must protest about the kinetic data presented for normal skin. Weinstein et al [2] calculate a value of 311 h for the cell cycle time, using the relationship:

$$T_c = \frac{T_s \times GF}{LI}$$

where T_s is the duration of the S phase, LI the percentage of labeled basal cells, and GF is the growth fraction (i.e., the proportion of the proliferative population which is actually cycling). Values for T_s obtained by different methods vary by a factor of at least 2-fold [5,6]. The measurement of LI is notoriously error-prone for technical reasons, published estimates again showing wide variation. Most important, however, is the choice of GF. As stated by Weinstein et al [2] just one single determination of GF has been reported using human skin in vivo [7]; a few additional data are available from xenografts of human skin onto nude mice. Based on this rather slender evidence, Weinstein et al select a value of 0.6 for their calculation of T_c.

We have recently quantified GF in healthy human skin using a flow cytometric approach. Following cellotape stripping, a distinct cohort of recruited G_0 cells was observed moving through the cell cycle between 36–56 h after injury. Quantification of this cohort by means of "windows" located at mid-S and G_2M yielded an estimate of 76% for the G_0 population, i.e., a value of 0.24 for GF (manuscript in preparation). Clearly this figure must be regarded as an upper limit since it assumes that all resting cells are mobilized in response to stripping. Using this value in combination with what we regard as best estimates for T_s and LI leads to a value of about 40 h for the cell cycle time in normal human epidermis; this is very different from the figure of 311 h proposed

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by Weinstein, and, in particular, does not differ from that found in psoriasis.

The discrepancy between our estimate of GF and that obtained using a continuous labeling technique could be explained in several ways. These include the assumption that a dynamic equilibrium exists between the G_0 population and the actively cycling pool of cells; alternatively, the nonphysiologic conditions for the determination of GF may induce some continuous recruitment. In both instances, the entire germinative population will eventually be labeled; it is clear that the "apparent" resting population will then depend on how long the period of observation is continued, but will always give an overestimate of GF.

We would emphasize that the distinction between a large population of slowly dividing cells or a small population of rapidly dividing cells is of more than merely academic interest. It is rapidly becoming clear that changes in growth rate resulting from injury from certain drugs (including corticosteroids) and in at least some skin diseases are mediated via alteration of GF and *not* via modulation of cell cycle times [6]. Failures to recognize this distinction will seriously impede our understanding of the molecular mechanisms underlying these phenomena.

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REPLY

Drs. Bauer and Boezeman, in their Letter to the Editor, agree with our analysis of cell kinetics in psoriasis, but disagree with our data on normal skin. They criticize our value for T_s (duration of the S phase) in normal skin found by the direct "frequency of labeled mitoses" (FLM) method that is the most commonly used technique in biologic systems to determine T_s. Our use of the FLM method in psoriasis for the T_s has been confirmed by others [1,2] and also leads to the cell cycle time (T_c) of 36 h that Bauer and Boezeman also confirm. For the value of T_s in normal skin, they refer in their letter to a review article by Gelfant which uncritically lists results from several old studies, and to their own work not published in a refereed journal for examination.

In our own article on normal epidermal kinetics [3] we too are concerned about labeling indices (LI), but find our data comfortably within the range of other published data. Bauer and Boezeman's major concern, as was ours, is the determination of a value for the growth fraction (GF) of normal skin. Direct data for human skin still remain minimal but the human data are backed up in part by high GF values of 80–100% in swine and mouse epidermis [4,5]. Swine epidermis appears to be a very good model for human epidermal kinetics with similar proven values for transit times [6] and LI.

In contrast to our information, Bauer and Boezeman are offering unpublished data utilizing a technique that nonphysiologically stimulates the skin with tape stripping. There is an obvious question of whether these data truly reflect the physiologic status of epidermal kinetics, G_0 epidermal cells, and whether flow cytometric techniques can accurately quantitate this cell population. Furthermore, the authors use their "best estimates" for T_s and LI in their calculations. What are these "estimates" and do they reflect a proven direct measurement for T_s as was used in our studies?

Their questions concerning a large population of slowly dividing cells versus a small population of rapidly dividing cells is very real, but the weight of currently available evidence, both direct and indirect, suggests that normal epidermal cells have a long cell cycle in contrast to psoriatic cells. Speculations on these theories will best be helped by additional data.

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